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Pyrolisis-MS and FT-IR analysis of fresh and decomposed dairy manure

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Abstract

The ability to predict manure nitrogen mineralization is essential for optimizing crop growth while preventing N losses to the environment. However, estimating mineralizable manure N is problematic because of the wide variety of organic manure N forms, as well as the lack of a rapid standardized method. Fourier-transformed infrared spectroscopy (FT-IR) is a promising technology, since it can detect the absorbance of proteins and primary amines, in addition to being instantaneous and non-destructive. Likewise, analytical pyrolysis-mass spectrometry (Py-GC/MS) has been used to study lignin and protein composition of forages. We carried out a 10-week laboratory incubation of manure-amended soil in order to test the sensitivity of FT-IR and Py-GC/MS to detect changes in manure during decomposition in soil. Four different dairy or beef manures were included. The manures (0.15 mg manure-N g^{-1} soil) were placed inside mesh bags during the soil incubation to allow for the recovery and analysis of fresh as well as decomposed manure. Infrared spectroscopy of the fresh and incubated manures showed that this technique is sensitive to changes in manure organic N after soil application. Bands associated with primary amines decreased during the incubation, while bands associated with proteins increased during manure decomposition. Bands associated with fatty acids tended to decrease during the incubation, possibly due to utilization as C sources. The spectroscopic data also showed that lignin-specific signals increase during manure decomposition, suggesting that the relative amount of lignin in manure increases as it decomposes in soil. The changes in peak areas of pyrolyzates were related to changes in manure constituents during decomposition. The Py-GC/MS data showed qualitative changes in manure lignin during decomposition. The relative amounts of nitrogen containing pyrolyzates, such as phenylacetonitrile and methylindole, changed during the decomposition, but the changes were not consistent across manures. In summary, this experiment showed that the use of manure bags is valuable in discerning between the N cycling dynamics of manure and soil, and that infrared spectroscopy, as well as Py-GC/MS show potential as analytical tools to study manure decomposition in soil.

1. Introduction

Animal manure can be an important amendment in sustainable agricultural systems by providing nutrients for crops as well as increasing soil organic matter. The beneficial effects of manure can be seen for decades after a regime of manure application [1]. Recently, the negative environmental impacts of manure mismanagement have received notoriety. Manure NO_3 is prone to leaching and can be an important source of pollution to groundwater [2,3]. To achieve a balance of agronomic benefit and minimal environmental pollution, it is necessary to have accurate estimates of the potentially available nutrients in manures and use this knowledge to make correct management decisions.

Animal manure is made up of a diverse set of components including partially digested feed, cells and bedding, each with different mineralization properties ranging from recalcitrant lignin to labile N pools [4]. Because of this,

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some manures will readily provide N to crops, whereas other manures may take months to supply N due to the slow mineralization of organic manure N [5–8].

The traditional method of estimating the N mineralization potential of manure is by aerobic incubation in soil and analysis of mineralized N. Incubations are slow to produce results, making them unsuitable for timely decisions about the rates of manure application. The results from incubations are not easily replicated because of the hard to control incubation conditions, soil type, moisture, aeration, as well as differences in the way the manure is mixed with the soil. In addition, denitrification losses during incubations may affect the estimates of the N supplying capacity of manure [9,10].

Manure components that reliably predict potentially mineralizable manure N have not yet been identified. Previous work has shown that manure properties like volatile fatty acids, lignin, C/N ratios and total N content are not reliable predictors of mineralizable N in manure [10]. The ideal manure mineralizable N assay should be a rapid instrumental method that can be readily standardized for use in different laboratories and is portable for use in the field. As a first step in the design of an appropriate protocol, data is needed about compositional changes in manure after application to soil.

Infrared spectroscopy is a promising technology, since it can discern between different N functional groups in organic matter, in addition to being instantaneous and non-destructive. Infrared spectroscopy has been used to measure the relative amounts of lignin, carbohydrate and protein in animal feeds [11]. Mid-infrared spectrometry has been used to develop calibrations for fiber, C and N content in manures [12]. A useful feature of infrared spectroscopy is that it can be used to analyze changes in compounds, such as recalcitrant lignins and labile carbohydrates, which can indirectly affect N mineralization by affecting C availability.

Analytical pyrolysis has been used to characterize the structure of organic N compounds in soils [13–15], as well as protein and fiber content in ruminant feedstuffs and corn stover [16–20]. The technique allows for sensitive, fast and precise identification of N-containing pyrolysis products.

The objective of this study was to carry out a qualitative assessment of manure degradation during decomposition in soil by using FT-IR and Py-GC/MS. Four different cattle manures were incubated in soil for 10 weeks. The manure was enclosed in mesh bags to allow for the FT-IR and pyrolysis-MS analysis of fresh manure as well as manure in different stages of decomposition.

2. Experimental

The Beltsville silt loam soil (fine-loamy, mixed, mesic Typic Fragiudults) was obtained at the Beltsville Agricultural Research Center from 0 to 10 cm depth and was sieved (4.76 mm mesh) before the experiment. The soil character-

istics were determined at the University of Maryland Cooperative Extension Laboratory: 5.7 pH (saturated paste method), 40.0 mg g^{-1} organic matter content, 60% sand, 27% silt and 13% clay. Total soil C (1.98%) and N (0.16%) were determined on dried (55 °C) samples with a CNS-2000 LECO Elemental Analyzer (LECO Corporation, St. Joseph, MI).

There were four manure types: (1) UMB-obtained from Hereford-Angus steers fed with Timothy hay and prepared feed (see below). (2) USB-collected from Hereford-Angus heifers allowed to graze and supplemented with prepared feed. (3) USD-obtained from a confined milking herd of Holstein cows on a protein-rich diet. (4) USH-obtained from a herd of Holstein heifers on a diet of prepared feed. All manures were obtained on September 2002 and stored at 5 °C. All manures except the USD were free of urine. Care was taken to exclude any soil or bedding materials from the manures. In order to homogenize each manure, they were mixed with dry ice (1:2, v/v) and blended at high speed in a Vita Mix 3600 Plus blender (Vita Mix Corp., Cleveland, OH). The manures were stored overnight at 5 °C until all the CO_2 evaporated. The manures were then placed at -20 °C until the loading of the manure bags. The total C content (fresh weight basis) ranged from 8.45% in UMB to 7.05% in USB, while the C/N ratio of the manures ranged from 22.2 in UMB to 16.15 in USD.

Feed samples were collected as presented to the animals. The feeds were different for each treatment, and were composed of mixtures of grain, meal, silage, molasses, seed, fruit, salt, minerals, calcium carbonate and vitamins. The feeds were dried at 60 °C and ground before the FT-IR and Py-GC/MS analyses.

The mesh bags were made of polyethylene/polyester filter material (Unibond HK-250-N, Midwest Filtration Co., Fairfield, OH), welded with a Kwik Seal sealer (Cole Parmer Instrument Co., Chicago, IL). This filter material is an amorphous open cell filtration medium, and the rate of movement of water through the material is equivalent to a 0.5 μm filter. The bags retained the manure solids while allowing for the exchange of fluids between the manure and the soil. The manure added to each bag amounted to 0.15 mg manure-N g^{-1} soil. The fresh weight added to each microcosm varied from 3.73 to 3.96 g depending on the manure N content.

Each microcosm consisted of a manure bag buried in 125 g of soil inside a 120 mL specimen cup. Water was added to all microcosms to achieve 25% gravimetric moisture. The microcosms were then put in 3.8 L jars, incubated aerobically, and destructively sampled at 0, 1 2, 4, 7 and 10 weeks. At each sampling, the manure was removed from the bag and stored at -20 °C until analysis by FT-IR and Py-GC/MS.

2.1. Infrared spectroscopy

Before the Py-GC/MS and the FT-IR analyses, the manures were freeze-dried in the bags, and the lyophilized

manures were stored in a desicator at room temperature. The homogenization with dry ice resulted in fine manure solids, so the manures did not need to be ground after lyophilization. Lyophilized samples were scanned in the mid-infrared from 4000 to 400 cm^{-1} (2500–25,000 nm) on a DigiLab FTS 7000 FTIR spectrometer, using KBr as background reference.

2.2. Py-GC/MS

Duplicate subsamples (1 mg each) of lyophilized manure were pyrolyzed at 600 °C for 30 s using a AS-2500 Pyrolysis Autosampler (Chemical Data systems, Oxford, PA) fitted with a quartz pyrolysis chamber surrounded by a platinum coil. The pyrolyzer was interfaced at 200 °C with a GCQ Benchtop Ion Trap GC/MS (Thermo Finnigan, San Jose, CA). The operating conditions were as follows: splitless injection, injector temperature at 300 °C, J&W DB-5 ms column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA). The temperature

program started at 50 °C (held for 10 min) and increased at 5 °C min^{-1} to 300 °C (held for 10 min). The He carrier gas was set at a constant velocity of 40 cm s^{-1}. The transfer line temperature was 300 °C, and mass spectral scan from 40 to 650 m/z, recorded (1 scan s^{-1}) at 70 eV. The peak identification was carried out by comparison of each peak's mass spectrum to library spectra [21].

2.3. Principal components analysis

We used the proc PRINCOMP procedure of SAS Version 8.02 (Cary, NC, USA) in order to illustrate the changes in the FT-IR spectra and Py-GC/MS during manure decomposition. All spectral data were converted to percent of total absorbance, and all Py-GC/MS data was converted to percent of total peak height. All principal components were computed from the covariance matrix, since all the data used for each principal components analysis (PCA) had the same scale. A total of 35 pyrolyzates were used to carry out the PCA analysis (Table 1). The PROC CORR of SAS Version

Table 1
Identification of Py-GC/MS products of manures and feeds

	Pyrolyzate	R_t (min)	Ions $(m/z)^2$
1	Unknown 1	2.8	43, 61, 75, 45, 41
2	Toluene	4.5	91, 92, 65, 39, 63, 51
3	Furfural	6.1	96, 95, 67, 42
4	Unknown 2	7.5	43, 55, 60, 91, 106, 81
5	Unknown 3	8.6	43, 78, 104, 42, 55
6	Unknown 4	11.6	41, 55, 70, 42, 43
7	Phenol	15.3	94, 66, 39, 65
8	Unknown 5	15.8	67, 91, 79, 80, 77, 93
9	Unknown 6	16.8	84, 112, 41, 55, 42, 91
10	Unknown 7	17.8	77, 108, 79, 107, 41, 89
11	Guaiacol + methylphenol[a]	18.8	129, 124, 81 and 107, 108, 77, 79, 80, 51
12	Phenylacetonitrile	20.0	90, 117, 89, 116
13	Unknown 8	20.2	126, 43, 55, 83, 97, 41
14	A dimethylphenol	21.3	107, 122, 77, 103
15	Methylguaiacol	22.2	138, 123, 95, 67
16	Unknown 9	23.0	43, 41, 57, 55, 42
17	Vinylphenol	24.0	51, 65, 91, 120
18	Ethylguaiacol	25.2	137, 152, 122, 91, 77
19	Vinylguaiacol	26.3	150, 135, 77, 107
20	Eugenol	27.4	164, 77, 103, 149, 133
21	Syringol	27.4	154, 65, 93, 139, 96, 111
22	Methyl indole	28.2	130, 131, 77, 103, 51
23	cis Isoeugenol	28.4	125, 180, 91, 41, 69
24	Vanillin	28.7	151, 152, 109, 53, 65, 137
25	Methylsyringol + trans isoeugenol[a]	29.9	168, 107, 153, 125 and 164, 149, 77, 103, 131, 121
26	Unknown 10	30.6	55, 41, 69, 67, 83, 97
27	Unknown 11	30.8	41, 57, 43, 71, 85
28	Ethylsyringol	31.7	167, 182, 79, 107, 137, 151, 95
29	Vinylsyringol	32.8	180, 165, 137, 91, 77, 122
30	Allylsyringol	33.6	194, 77, 119, 179, 131, 103, 147
31	cis Propenylsyringol	34.8	194, 119, 179, 77, 131, 151
32	trans Propenylsyringol	36.0	194, 119, 179, 77, 131, 151
33	Unknown 12	36.3	41, 55, 111, 70, 69
34	Unknown 13	38.7	67, 95, 81, 41, 55, 123
35	Unknown 14	39.5	67, 81, 95, 79, 41

All are unconfirmed library search results. Unknowns are unresolved molecules or mixtures of unknown molecules.
  [a] Unresolved mixture of methylsyringol and trans isoeugenol.

8.2 (Cary, NC) was used to determine the Pearson correlation coefficients (r) between the spectral data and time of incubation.

3. Results and discussion

3.1. Manure and feed FT-IR spectra

Fig. 1 shows the FT-IR spectra of the feeds and hay. The feeds and hay show the characteristic peaks for amines and OH functional groups (3400 cm^{-1}), C–H bonds (2870 cm^{-1}), esters from carbohydrates (1734 cm^{-1}), amides in proteins (1653 cm^{-1}), aromatic rings in lignin (1510 cm^{-1}) and cellulose (897 cm^{-1}). In this work, we assign absorbances to functional groups according to information from previous work done with materials of known composition. However, very few compounds of the nature we are discussing have baselines of zero absorption in the 1700–400 cm^{-1} region, and it is possible that other compounds have a small influence on the spectra besides protein, lignin and cellulose. The hay has the most prominent cellulose and lignin peaks. The UMB feed lacks a cellulose peak possibly because it is grain-based. It is not contradictory that a material that haves prominent lignin peaks lacks an ester peak at 1734 cm^{-1}. Lignin composition varies widely in plant material, and different lignins can have different amounts of esters. Purified lignin from peanut
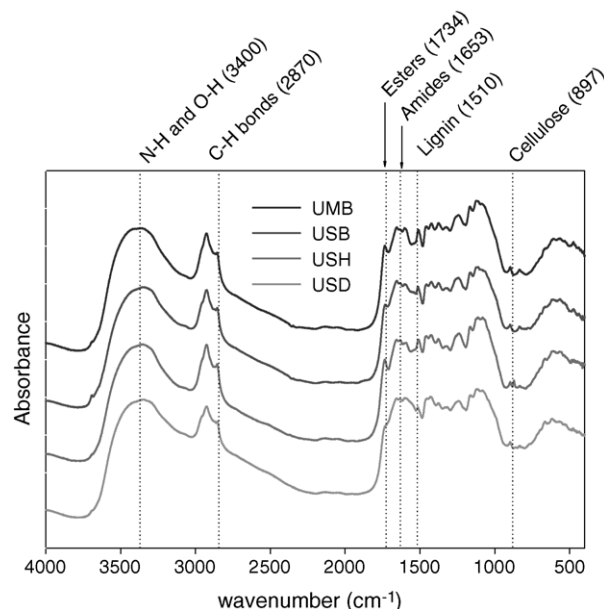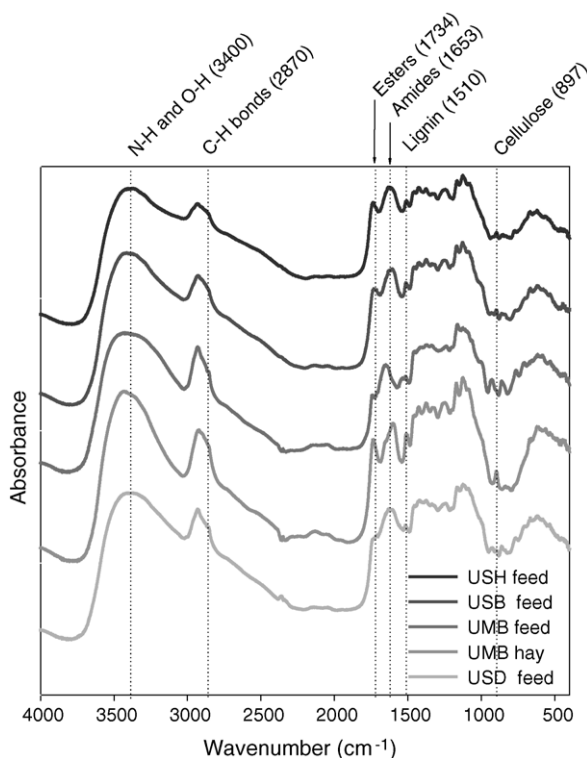

Fig. 1. FT-IR spectra of the feeds and hay. The feeds are mixtures of grains, hay, silage, minerals and other supplements, while the hay is made up of timothy hay exclusively.


Fig. 2. FT-IR spectra of the fresh manures (week 0).

hulls may have prominent peaks at 1507 cm^{-1} also 1601 and 1659 cm^{-1}, and a weak, peak for esters (1 L). Like the feeds, the FT-IR spectra of the fresh manures show the distinctive absorptions for amines and OH groups, C–H bonds, carbohydrates, proteins, lignin and cellulose (Fig. 2).

3.2. Comparison between feed and manure FT-IR spectra

Fig. 3 shows the subtracted spectra of the USD and USH fresh manures and feeds in order to show changes during digestion. Only the USD and USH were included, since the animals in these treatments were not allowed to graze, and fed exclusively on the prepared feed, which allows us to assume that only the material in the feed was consumed by the animals. Compared to the feeds, the manures had higher absorbance at several diagnostic wavelengths. Bands at 2870 cm^{-1} (C–H groups), 1653 cm^{-1} (amides in proteins), 1510 cm^{-1} (aromatic absorption from lignin), 1083 cm^{-1} (tertiary C–N, C–O, aromatic C–C groups) and 897 cm^{-1} (cellulose) increase markedly during digestion. Time zero manures had higher absorbance than the feeds between 3100 and 3600 cm^{-1}, which corresponds to N–H and O–H groups. The shape of the subtracted peak suggests that proportionately more OH absorbance than NH absorbance was lost after the feed was digested by the animals.

Table 2 shows the ratio of absorbance at specific wavelengths in feeds and fresh USD and USH manures in order to illustrate the spectral changes of the feed as it is digested and converted to manure. In both the USD and USH manure treatments, lignin peaks increase relative to amines/OH, cellulose, C–H bonds, as well as carbohydrate during the digestion, showing that lignin increases in feeds as they pass through the digestive system. Protein-specific bands
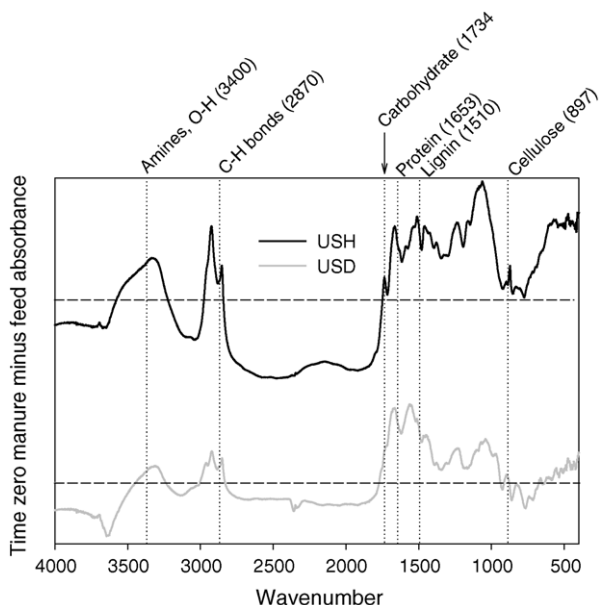
Fig. 3. Substracted spectra of the manures and feeds. Two manures are shown: USD and USH.

(amides) increase relative to C–H bonds, cellulose and esterified carbohydrates during feed digestion in the animal. Besides protein and lignin bands, esterified carbohydrate bands decrease during the digestion relative to cellulose bands. The band at 2870 cm^{-1}, associated with C–H bonds, decreases relative to most other wavelengths included in both manures. In both USD and USH, fresh manure absorbed more at 1083 cm^{-1} than the corresponding feed. This band includes aromatic C–C groups, which could be

attributed to lignins or proteins. Also, fresh manures have higher absorbance than feeds at 1734 cm^{-1}, specific for esterified carbohydrates, and at 2870 cm^{-1}, specific for C–H groups. Lignins have esters, so it is difficult to interpret whether the 1734 cm^{-1} band belongs to the relatively easy to digest carbohydrates, or from a recalcitrant lignin. However, given the finding that absorption at 1734 cm^{-1} increases after digestion, we speculate that some of the absorption at this wavelength is due to lignin. Straws are rich in CH groups, suggesting that this type of material is relatively resistant to digestion. In addition, fatty acids and amino acids are also CH-rich, suggesting that these two substances increase in concentration during digestion.

3.3. Changes in manure FT-IR spectra during decomposition in soil

Fig. 4 shows the series of spectra according to the weeks of incubation for the USD manure. Only the USD manure is included, since it illustrates what happened to the rest of the manures. Bands associated with primary amines (1340–1020 cm^{-1}), lignin (1510 cm^{-1}), as well as amides associated with proteins (1653 cm^{-1}) increase as the USD manure decomposes in soil. The increase in the protein band in manure during decomposition may be due to microbial growth and N immobilization within the manure as C is mineralized and lost. Bands associated with fatty acids, such as C–H (2870 cm^{-1}) and carboxylic acids (3000–2500 cm^{-1}) tend to decrease during the decomposition, possibly due to utilization as C sources. The spectroscopic data also shows that lignin-specific signals (1600–1590 and 1510 cm^{-1}), change during manure decomposition,

Table 2
Ratio of absorbance at selected spectral bands between feed spectra and fresh manure

Ratio[a]	USD feed	USD manure	USH feed	USH manure
Amines, –OH:C–H bonds	1.19	1.17	1.14	1.18
Amines, –OH:carbohydrates	1.29	1.22	1.08	1.12
Amines, –OH:amides in proteins	1.12	0.99	1.03	0.97
Amines, –OH:lignin 1	1.09	0.99	1.01	0.99
Amines, –OH:lignin 2	1.20	1.06	1.07	0.97
Amines, –OH:cellulose	1.40	1.40	1.30	1.35
C–H bonds:carbohydrates	1.09	1.04	0.95	0.95
C–H bonds:amides in proteins	0.94	0.84	0.90	0.82
C–H bonds:lignin 1	0.92	0.84	0.89	0.84
C–H bonds:lignin 2	1.01	0.90	0.94	0.82
C–H bonds:cellulose	1.17	1.19	1.15	1.14
Carbohydrates:amides in proteins	0.87	0.81	0.95	0.87
Carbohydrates:lignin 1	0.85	0.81	0.93	0.89
Carbohydrates:lignin 2	0.93	0.87	0.99	0.87
Carbohydrates:cellulose	1.08	1.15	1.20	1.20
Amides in proteins:lignin 1	0.98	1.00	0.98	1.02
Amides in proteins:lignin 2	1.07	1.07	1.04	1.00
Amides in proteins:cellulose	1.25	1.41	1.27	1.39
Lignin 1:lignin 2	1.10	1.07	1.06	0.98
Lignin 1:cellulose	1.27	1.42	1.29	1.36
Lignin 2:cellulose	1.16	1.32	1.22	1.39

[a] Amines + OH band = 3400 cm^{-1}, C–H bonds band = 2870 cm^{-1}, esterified carbohydrates band = 1734 cm^{-1}, amides in proteins band = 1653 cm^{-1}, lignin 1 band = 1600 cm^{-1}, lignin 2 band = 1510 cm^{-1} and cellulose band = 897 cm^{-1} [11].

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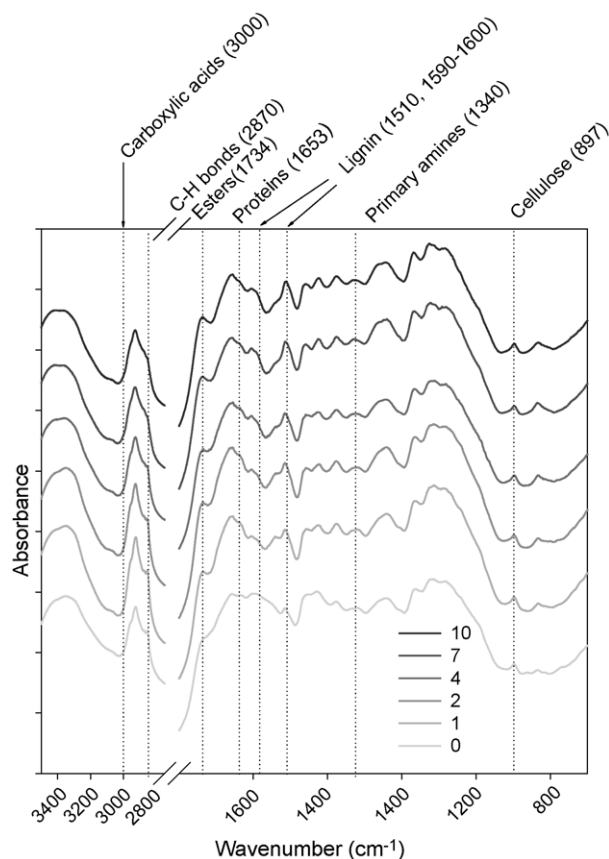
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Fig. 4. FT-IR spectra of the fresh USD manure (time 0), as well as manures incubated in soil for 1, 2, 4, 7 and 10 weeks. The Y-axis has been made unit less in order to be able to stack the graphs.

suggesting a change in lignin structure, while cellulose-specific bands increase.

A comparison of week 10 and fresh USD manures shows the net effect of manure decomposition on the FT-IR spectra (Fig. 5). Decomposed manures had lower absorbance than the fresh manure at 3400 cm^{-1} (N–H and O–H groups) and 2870 cm^{-1} (C–H groups). Fatty acids and proteins are rich in C–H groups, while cellulose is not. Our results suggest that there was a preferential utilization of fatty acids and/or proteins relative to cellulose during decomposition in soil. In contrast the decomposed manures had higher absorbance at the 1653 cm^{-1} (proteins), 1510 cm^{-1} (lignin) and 897 cm^{-1} (cellulose). The effect of decomposition on absorbance at 1734 cm^{-1} (esterified carbohydrates) was mixed, since there was a net increase in the USD manure, but a slight decrease in the rest of the manures (data not shown).

Table 3 shows how specific wavelengths change relative to each other as the manure decomposes in soil. In all four manure treatments, the C–H bond band decreases relative to all other bands during decomposition, showing that manure components rich in C–H bonds, such as fatty acids decrease during decomposition in soil. The band specific for amides in proteins decreases relative to cellulose, increases relative to the amine + OH band, and shows no consistent pattern relative to the carbohydrate band. Carbohydrate bands
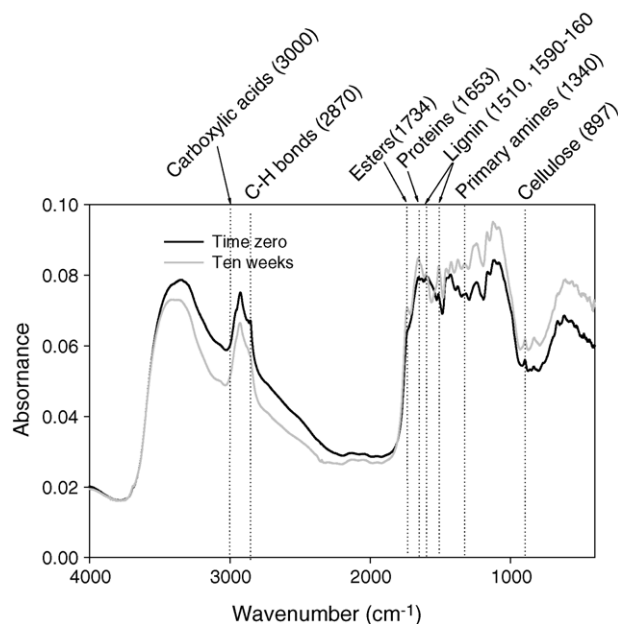

Fig. 5. FT-IR spectra of fresh USD manure (time 0) and USD manure incubated 10 weeks in soil.

decrease during decomposition relative to cellulose bands. The two bands associated with lignin differ in their changes relative to other bands with the exception of an increase in absorbance of both lignin bands relative to the amines + OH band.

3.4. Multivariate analysis of FT-IR spectral data from fresh and decomposed manures

The PCA plot shows that there are chronological differences between the manure spectroscopic data (Fig. 6). While the different incubation times did not form distinct groupings, it is evident that manures after 7 weeks of incubation have different spectra from manures with 4 weeks or less of decomposition. Component two separates early from later sampling times, since fresh manures and manures with short periods of decomposition have high values along this axis. Fig. 7 shows the correlation of absorbance at specific wavelengths with weeks of decomposition. There was a high positive correlation (correlation coefficient >0.90) between weeks of decomposition and wavelengths between 743 and 781 cm^{-1}. These wavelengths include bands specific for amines, amides, alcohols and aryl-NH$_2$ groups. There was a high negative correlation between time of decomposition and wavelengths between 2870 and 2905 cm^{-1}, which include specific bands for C–H bonds, carboxylic acids, N–H and O–H groups.

3.5. Comparison between feed and manure pyrolyzates

Fig. 8 shows the subtracted fresh manure minus feed Py-GC/MS data. Analytical pyrolysis allows for the gas chromatographic analysis of manure components of high

Table 3
Ratio of absorbance at selected spectral bands between fresh manure and manure incubated for 10 weeks

Ratio[a]	USD week 0	USD week 10	USH week 0	USH week 10	USB week 0	USB week 10	UMB week 0	UMB week 10
Amines, –OH:C–H bonds	1.17	1.24	1.18	1.22	1.18	1.21	1.20	1.24
Amines, –OH:carbohydrates	1.22	1.03	1.12	1.10	1.13	1.04	1.13	1.01
Amines, –OH:amides in proteins	0.99	0.86	0.97	0.86	0.96	0.81	1.00	0.81
Amines, –OH:lignin 1	0.99	0.91	0.99	0.90	0.98	0.84	1.00	0.84
Amines, –OH:lignin 2	1.06	0.89	0.97	0.86	1.00	0.84	1.01	0.84
Amines, –OH:cellulose	1.40	1.18	1.35	1.17	1.34	1.12	1.35	1.09
C–H bonds:carbohydrates	1.04	0.83	0.95	0.90	0.96	0.86	0.94	0.82
C–H bonds:amides in proteins	0.84	0.70	0.82	0.70	0.82	0.66	0.83	0.66
C–H bonds:lignin 1	0.84	0.74	0.84	0.73	0.83	0.69	0.83	0.68
C–H bonds:lignin 2	0.90	0.72	0.82	0.70	0.85	0.70	0.84	0.68
C–H bonds:cellulose	1.19	0.95	1.14	0.96	1.14	0.93	1.12	0.87
Carbohydrates:amides in proteins	0.81	0.84	0.87	0.79	0.85	0.77	0.88	0.80
Carbohydrates:lignin 1	0.81	0.88	0.89	0.82	0.86	0.81	0.88	0.83
Carbohydrates:lignin 2	0.87	0.86	0.87	0.79	0.88	0.81	0.89	0.83
Carbohydrates:cellulose	1.15	1.14	1.20	1.07	1.18	1.08	1.19	1.07
Amides in proteins:lignin 1	1.00	1.06	1.02	1.04	1.02	1.04	1.00	1.04
Amides in proteins:lignin 2	1.07	1.03	1.00	1.00	1.04	1.05	1.02	1.04
Amides in proteins:cellulose	1.41	1.37	1.39	1.36	1.40	1.39	1.36	1.33
Lignin 1:lignin 2	1.07	0.97	0.98	0.96	1.02	1.00	1.01	1.00
Lignin 1:cellulose	1.42	1.29	1.36	1.31	1.37	1.34	1.35	1.29
Lignin 2:cellulose	1.32	1.33	1.39	1.36	1.34	1.33	1.34	1.29

The standard deviation of the ratios for all wavelenghts ranged from 2.48×10^{-4} to 4.51×10^{-3}.

[a] Amines + OH band = 3400 cm^{-1}, C–H bonds band = 2870 cm^{-1}, esterified carbohydrates band = 1734 cm^{-1}, amides in proteins band = 1653 cm^{-1}, lignin 1 band = 1600 cm^{-1}, lignin 2 band = 1510 cm^{-1} and cellulose band = 897 cm^{-1} [11].

molecular weight. The resulting pyrogram includes contributions from manure constituents, such as lignins and proteins. Thus, the concentration of manure components is reflected in the number and area of the peaks originating from such components. Several pyrolyzates increase during the digestion including: unknown 1, vinylphenol, vinylguaiacol, eugenol, syringol, methyl indole, *cis* isoeugenol, vanillin, methylsyringol + *trans* isoeugenol, unknown 10,

ethylsyringol, vinylsyringol, allylsyringol, *cis* propenylsyringol, *trans* propenylsyringol and unknown 12. Other pyrolyzates decrease during digestion: toluene, furfural, unknown 2, unknown 3, unknown 4, phenol, unknown 6, unknown 7, guaiacol + methylphenol, phenylacetonitrile, unknown 8, a dimethylphenol, ethylguaiacol and unknown 13. Phenol, guaiacol + methylphenol, dimethylphenol and ethylguaiacol are lignin-derived pyrolyzates that decrease during digestion. However, the rest of the lignin-derived
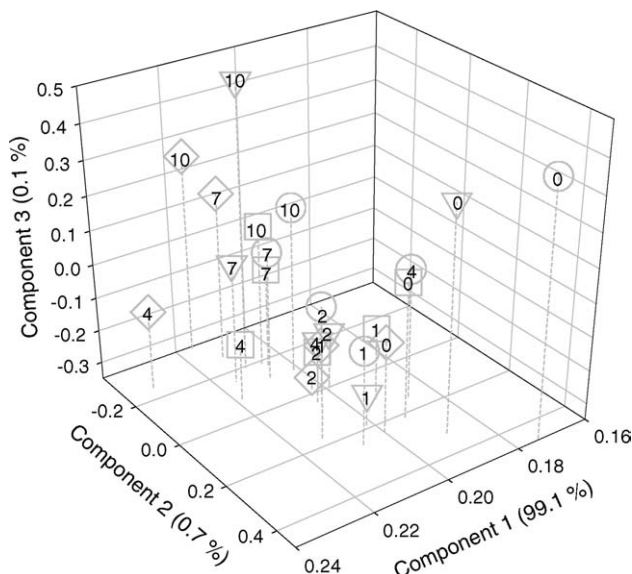

Fig. 6. Principal components analysis of the FT-IR spectra of fresh manures, as well as manures incubated for 1, 2, 4, 7 and 10 weeks in soil. Four different manures are shown: USD (circles), USH (squares), USB (triangles) and UMB (diamonds).
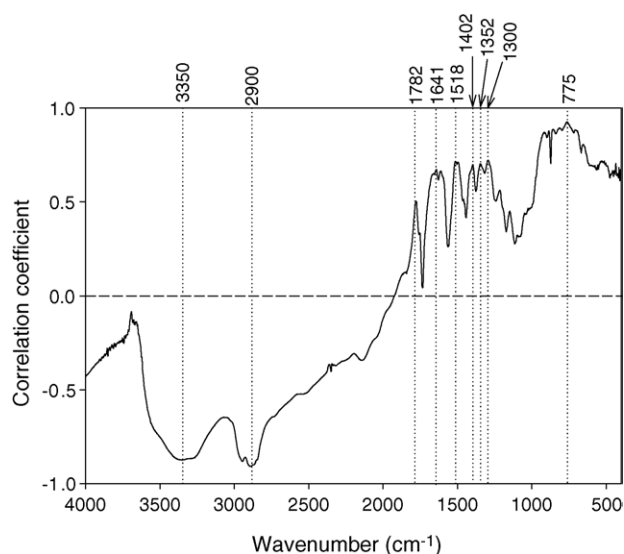

Fig. 7. Correlation coefficients for each FT-IR wavelength against time of decompositon. The data from four different manures was combined to generate the correlations.
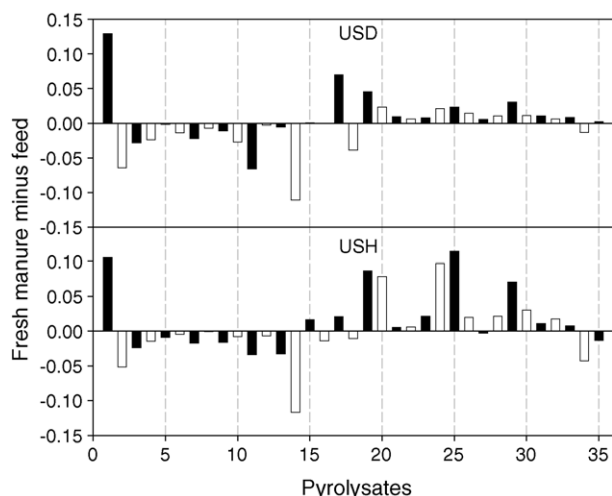
Fig. 8. Substracted pyrolyzates between fresh manure (time 0) and feed. The x-axis nubers correspond to the first column in Table 1.

pyrolyzates increase during digestion. Furfurals are derived from carbohydrates (14) and the Py-GC/MS data seems to contradict the FT-IR data, which shows slight increases in carbohydrate bands during conversion from feed to manure. However, it is important to note that the band at 1739 cm^{-1} could come not just from carbohydrate, but also from lignin esters and hemicellulose. Nitrile pyrolyzates are derived from proteins (14). Phenylacetonitrile declined during digestion, indicating uptake or modification by the animal. Similarly to the furfural, the phenylacetonitrile data seems to contradict the FT-IR results, where the protein band increased during digestion. Vinylguaiacol may be derived from lignin or protein, and it increased during digestion in both the USD and the USH treatments.

3.6. Changes in manure pyrolyzates during decomposition in soil

Fig. 9 illustrates the pyrograms of the fresh (week 0) and decomposed manures from the USD treatment. Many peaks
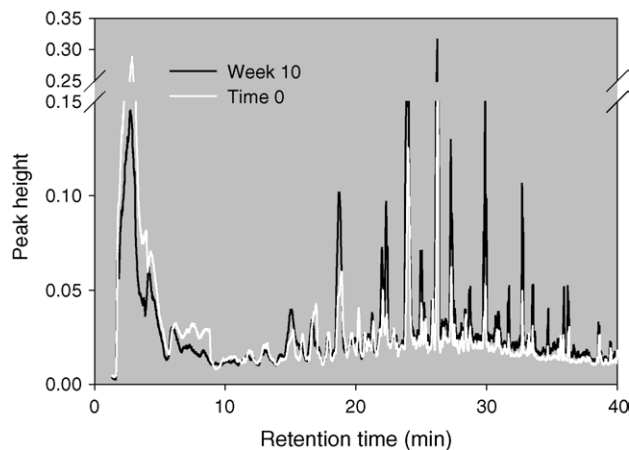
are comprised of lignin pyrolyzates, such as phenol, guaiacol + methylphenol, a dimethylphenol, methylguaiacol, vinylphenol, ethylguaiacol, vinylguaiacol, eugenol, syringol, *cis* isoeugenol, vanillin, methylsyringol + *trans* isoeugenol, ethylsyringol, vinylsyringol, allylsyringol, *cis* propenylsyringol and *trans* propenylsyringol [20]. As with digestion, the concentration of many lignin products increased during manure decomposition (Fig. 10). For most lignin-derived pyrolyzates, the changes were not consistent across manures. Exceptions are syringol and dimethylphenol, which increased in all the manures during decomposition. The changes in relative abundance of the different lignin products suggest a change in the lignin composition of the manures. As with the digestion results, the carbohydrate-derived furfural decrease contradicts the FT-IR data where a slight increase in the carbohydrate band was observed. We hypothesize that the Py-GC/MS is better suited than the FT-IR to detect changes in carbohydrate content in manure, since carbohydrates are readily available for microbial utilization in soil and are bound to decrease during manure decomposition. Phenylacetonitrile, a peak of protein origin,
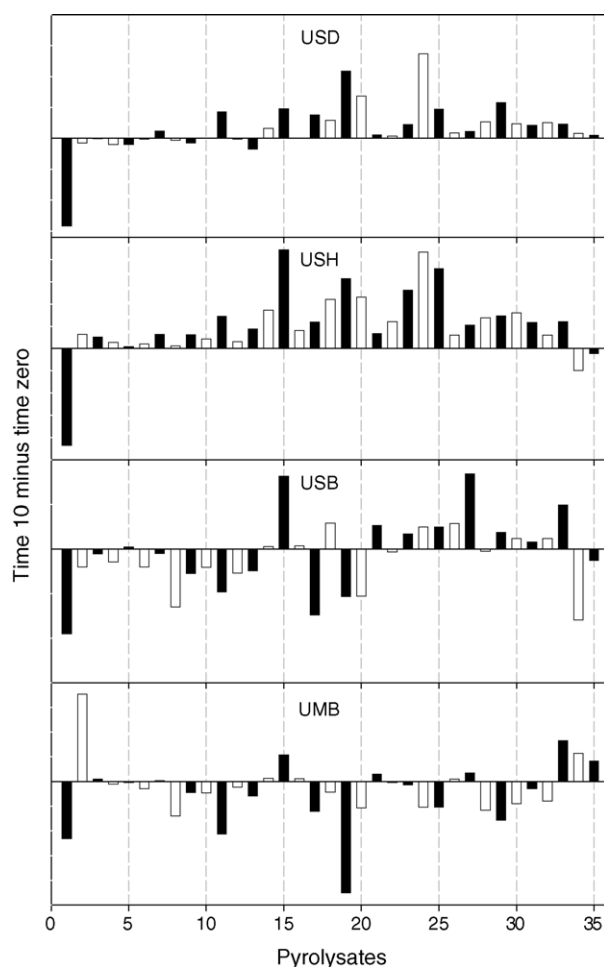

Fig. 9. Pyrogram of the fresh USD manure (time 0), and manure incubated for 10 weeks in soil.

Fig. 10. Substracted pyrolyzates between fresh manure (time 0) and manure incubated for 10 weeks in soil. The x-axis nubers correspond to the first column in Table 1.
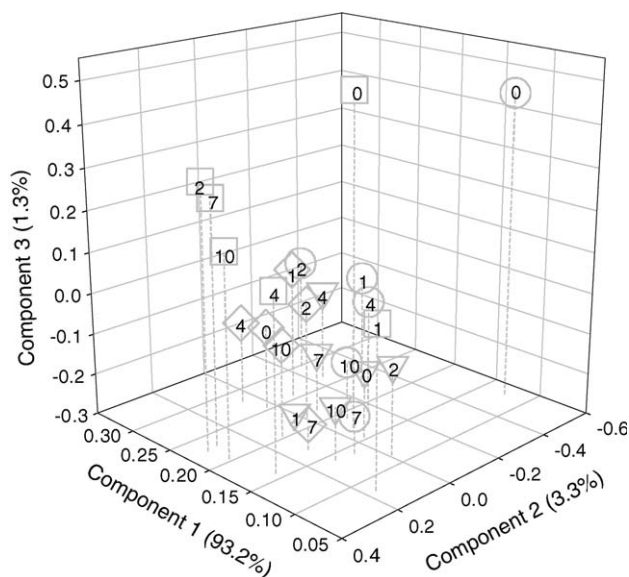
Fig. 11. Principal components analysis of the Py-GC/MS profiles of fresh manures, as well as manures incubated in soil. Four different manures are shown: USD (circles), USH (squares), USB (triangles) and UMB (diamonds).

had mixed results during manure decomposition, with slight decreases in two manures, and slight increases in the other two manures. Methylindole, also of protein origin, increased in USD and USH during decomposition, while little change was observed in the rest of the manures. Vinylguaiacol, derived either from lignin or protein, had mixed results with increases in USD and USH and decreases in USB and UMB. In contrast, the FT-IR data shows an increase in the protein band during decomposition in soil.

3.7. Multivariate analysis of Py-GC/MS data

The PCA of the pyrolysis data did not show a chronological separation of the manures (Fig. 11), indicating that decomposition does not result in a clear set of changes among pyrolyzate peaks. Likewise, we did not find any highly positive or negative correlations between any of the pyrolyzates and time of incubation (Fig. 12). The highest correlation coefficients were for methyl indole, dimethylphenol, unknown 12 and unknown 11, although all of them were below 0.68 (Fig. 12). It is possible that the initial variation between manures may have precluded high correlations between pyrolyzates and time of decomposition. However, the variation between manures did not prevent a chronological separation of the samples according to FT-IR spectra, showing that infrared spectroscopy is more sensitive to variations in manure composition than Py-GC/MS.

In conclusion, infrared spectroscopy data shows that the abundance of N containing functional groups changes during digestion and decomposition, showing that the technology is sensitive to fluctuations in manure N and
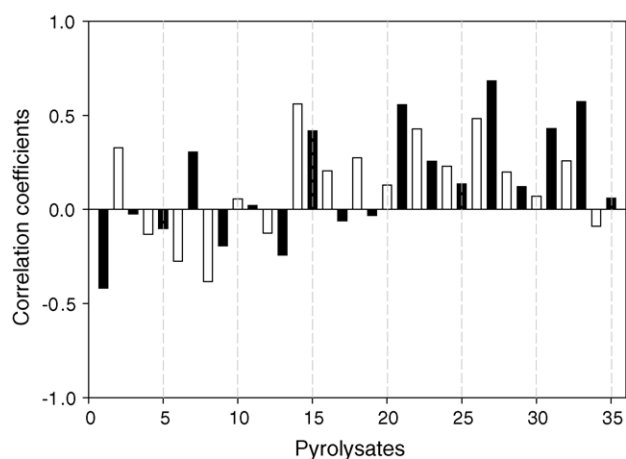

Fig. 12. Correlation coefficients for each pyrolyzate against time of decompositon. The data from four different manures was combined to generate the correlations.

lignin. Furthermore, we have shown that FT-IR profiles can be used to observe chronological changes in manure after soil application. Py-GC/MS data agreed with the spectroscopy data in that lignin quantity and quality change during digestion and decomposition. Further studies should be carried out to study apparent discrepancies between the two technologies regarding carbohydrate and protein changes during digestion and decomposition.

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